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# Structural Analysis of a Rare Rearranged Y Chromosome and Its Bearing on Genotype–Phenotype Correlation

**Tsutomu Ogata,[1]* Keiko Wakui,[2] Tomoki Kosho,[1] Koji Muroya,[1] Yasuko Yamanouchi,[3] Takako Takano,[3] Yoshimitsu Fukushima,[2] Gudrun Rappold,[4] Yoshimi Suzuki[5]**

*[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan*
*[2]Department of Hygiene and Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan*
*[3]Department of Hygiene and Public Health, Teikyo University School of Medicine, Tokyo, Japan*
*[4]Institute of Human Genetics, Heidelberg University, Heidelberg, Germany*
*[5]Department of Pediatrics, Toyohashi Municipal Hospital, Toyohashi, Japan*

**We report on a 9-year-old boy with a rare rearranged Y chromosome and borderline short stature (–2.0 SD). Standard metaphase chromosome analysis indicated a 46,X,i(Y)(q10) karyotype, but high resolution G-banding showed an asymmetric band pattern for the rearranged Y chromosome. FISH and DNA studies for a total of 15 different Y chromosomal loci or regions showed that the rearranged Y chromosome was accompanied by: 1) a partial deletion of the short arm pseudoautosomal region (PAR1) involving *SHOX*, with the breakpoint distal to *DXYS85*; and 2) a partial duplication of Yq, with the breakpoint proximal to *DAZ*. The karyotype was determined as 46,X,?i(Y)(q10).ish der(Y)(Yqter→Yp11.3::Yq11.2→Yqter)(DAZ++,DYZ3+,SRY+,SHOX–). The X chromosome and the autosomes were normal. The results suggest that haploinsufficiency of *SHOX* is primarily responsible for the borderline short stature, and that the deletion of the PAR1 may result in spermatogenic failure due to defective X-Y pairing and recombination in the PAR1. Am. J. Med. Genet. 92:256–259, 2000.**


**KEY WORDS: Y chromosome abnormality; structural determination; *SHOX*; pseudoautosomal region; short stature; spermatogenic failure**

## INTRODUCTION

The human Y chromosome has extensively been examined for its genetic function. To date, multiple genes including *SHOX* for short stature and Ullrich-Turner skeletal changes, SRY for gender determination, *USP9Y*/DFFRY for spermatigenic failure, and *SMCY* and *UTY* for H–Y antigens have been cloned from the Y chromosome [reviewed in Vogt et al., 1997; Kosho et al., 1999; Sun et al., 1999], and the genes for Ullrich-Turner syndrome (UTS) soft tissue and visceral stigmata, Y-specific growth control, gonadoblastoma, and azoospermia factor(s) have been mapped to specific regions of the Y differerntial region [reviewed in Vogt et al., 1997]. Furthermore, the Y chromosome has also been shown to play a pivotal role in spermatogenesis by pairing and recombining with the X chromosome in the short arm pseudoautosomal region (PAR1) [Chandley et al., 1984]. Thus, it has become possible to assess the clinical effects of rearranged Y chromosomes, if the precise structures have been determined. In this paper, we report on structural analysis of a rare rearranged Y chromosome, and discuss on genotype-phenotype correlation of the rearranged Y chromosome.

## CLINICAL REPORT

The male subject was born to healthy non-consanguineous parents at 40 weeks of gestation after an uncomplicated pregnancy and delivery. Birth length was 48.0 cm (–0.9 SD) and weight 3.3 kg (+0.2 SD). The postnatal course was uneventful, except for borderline short stature (around –2 SD) persisting from late infancy.

At age 8 6/12 years, the patient was seen at Toyohashi Municipal Hospital because of short stature. The

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*Correspondence to: Dr. Tsutomu Ogata, Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: t-ogata@po.iijnet.or.jp

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height was 116.5 cm (–2.0 SD) and the weight 23.0 kg (–0.7 SD). His bone age was assessed as 8 6/12 years; thus his predicted adult height (a child's final height as predicted from skeletal age of the child) was determined as 155.5 cm (–2.4 SD) by the method of Ito and Yokoya [1995]. The 40-year-old father was 160 cm tall and the 38-year-old mother 153 cm tall; thus his target height (a child's final height as predicted from the parental height) and target range (95% confidence interval of target height) were obtained as 165 cm (–0.8 SD) and 156~174 cm (–2.4 SD ~ +0.8 SD), respectively, by the equations of Ogata et al. [1990]. The 10 10/12-year-old sister was 137.4 cm tall (–0.5 SD), and the 7 6/12-year-old sister was 117.0 cm tall (–0.7 SD).

Physical examination at that time was unremarkable. He had no UTS somatic anomalies including short 4th metacarpals and cubitus valgus. External genitalia were those of prepubertal boys, with the testis size of 1–2 ml bilaterally (age-matched reference, 1.0 ± 0.2 ml) and the penile length of 6.0 cm (4.2 ± 0.6 cm) [Fujieda and Matsuura, 1987a, b]. Psychomotor development seemed to be age-appropriate. Routine laboratory tests were normal, as were endocrine studies for short stature. Bone survey showed no abnormal findings including Madelung deformity. At present, he is 9 3/12 years old, measures 120.5 cm (–2.0 SD), and shows no pubertal signs.

## Chromosome Analysis

The karyotype of the patient was initially interpreted as 46,X.i(Y)(q10) in 500 lymphocytes examined by G-banding and in 100 lymphocytes analyzed by Q-banding (Fig. 1A). High resolution G-banding, however, revealed an asymmetric band pattern for the rearranged Y chromosome; it seemed that most Yq portion was duplicated and attached onto the tip of Yp in an inverted direction (Fig. 1B). No abnormality was detected for the X chromosome or the autosomes. The parental karyotypes were normal in 20 lymphocytes analyzed.

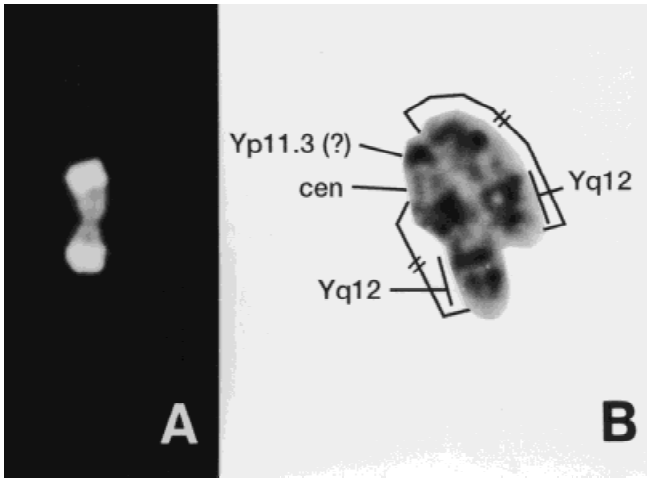


Fig. 1. The rearranged Y chromosome. (**A**) Q-banding appearance similar to an i(Yq) chromosome. (**B**) High resolution G-banding appearance showing an asymmetric band pattern, with an extra band reminiscent of Yp11.3.

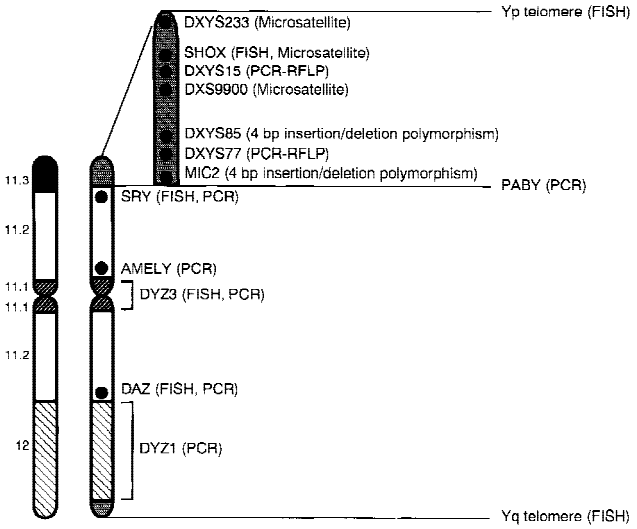


Fig. 2. The chromosomal location of examined loci or regions on the Y chromosome. The left-side ideogram shows the G-banding pattern of the Y chromosome. For the right-side diagram, the stippled, the white, the thick striped, and the thin striped areas represent the pseudoautosomal region, the Y-differential euchromatic region, the centromeric region, and the heterochromatic region, respectively. The methods used for the analysis of each locus are given in parentheses. The FISH probes used are cY29 for the Xp/Yp telomeric region [Ning et al., 1996], 34F5 for SHOX [Rao et al., 1997], pHu14 for SRY (Department of Genetics, Cambridge University), an α-satellite probe for DYZ3 (Oncor), a cosmid probe for DAZ (SRL), and c8.2/1 for the Xq/Yq telomeric region [Ning et al., 1996]. The PCR primer sequences for AMELY have been described in Nagafuchi et al. [1992], and those for the remaining loci have been reported in Genome Database.

## FISH Analysis

Probes defining six loci or regions on the Y chromosome were hybridized to lymphocyte metaphase spreads of the patient (Fig. 2). In addition, a Y-euchromatic region painting probe (pBS-Y) [Collins et al., 1991], a Yp painting probe (TA2401, ALT), and a Yq painting probe (TA2402, ALT) were hybridized to metaphase spreads. The probes were labeled with either digoxigenin or biotin; digoxigenin labeled probes were detected by rhodamine and anti-digoxigenin, and biotin labeled probes were detected by avidin and fluorescein isothiocyanate.

Representative results are shown in Figure 3. The Xp/Yp telomeric region and *SHOX* were absent from the rearranged Y chromosome, although they were detected on the normal X chromosome. *SRY* and *DYZ3* were present in a single copy on the middle part of the rearranged Y chromosome. *DAZ* and the Xq/Yq telomeric region were present in two copies on both arms of the rearranged Y chromosome in a nearly symmetrical position. The euchromatic region of the rearranged Y chromosome was homogeneously stained with the Y-euchromatic region painting probe. The middle part of the rearranged Y chromosome was stained with the Yp painting probe, and the remaining region was stained with the Yq painting probe.

## DNA Analysis

Seven loci on the PAR1 were determined for the copy number by PCR-based microsatellite, 4-bp insertion/deletion, and RFLP analyses, using leukocyte genomic

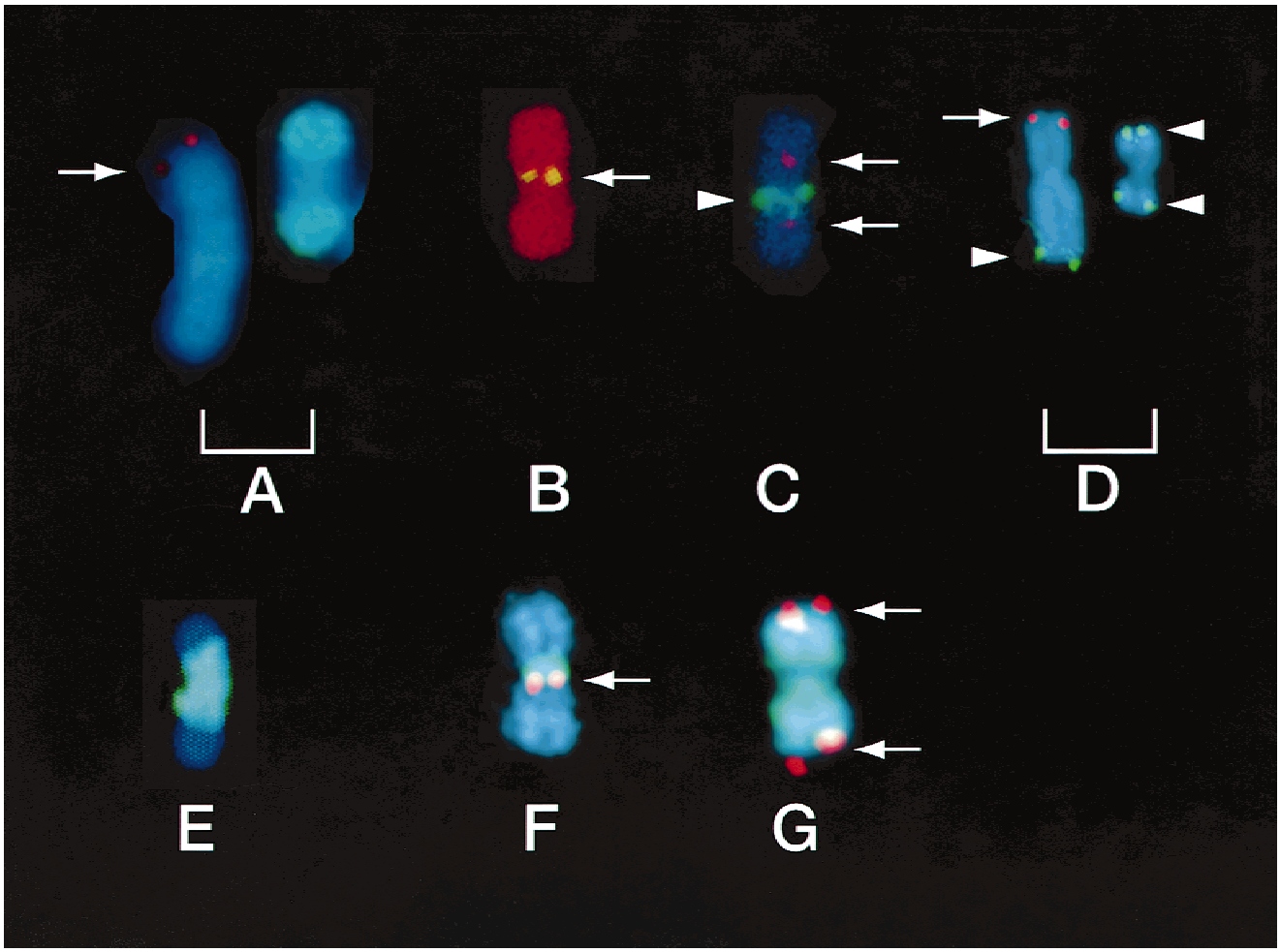


Fig. 3. FISH analysis in the patient. (**A**) SHOX. The positive signal is detected on the X chromosome (arrow) but is undetected on the rearranged Y chromosome. (**B**) SRY. The positive signal is delineated on the middle part of the rearranged Y chromosome (arrow). (**C**) DYZ3 and DAZ. The rearranged Y chromosome has a single copy of DYZ3 (arrowhead) and two copies of DAZ (arrows). (**D**) The Xp/Yp telomere and the Xq/Yq telomere regions. The X chromosome is positive for both the Xp/Yp telomere region (arrow) and the Xq/Yq telomere region (arrowhead), whereas the rearranged Y chromosome is negative for the Xp/Yp telomere region and has two copies of Xq/Yq telomere region (arrowheads). (**E**) Y-euchromatic painting. The euchromatic region of the rearranged Y chromosome is homogeneously stained. (**F**) Yp painting and DYZ3. The middle part of the rearranged Y chromosome is painted, and DYZ3 is detected on the one end of the painted region (arrow). (**G**) Yq painting and the Xq/Yq telomere region. Most of the rearranged Y chromosome, except for its middle part, is painted, with two positive signals for the Xq/Yq telomere region (arrows).

DNA of the patient and the parents (Fig. 2). For microsatellite and 4 bp insertion/deletion analyses, 0.3 μg of DNA was amplified by PCR with a fluorescently labeled forward primer and an unlabeled reverse primer, and the PCR products were determined for the product size and area under curve on an autosequencer (ABI PRISM™ 310) using GeneScan™. For PCR-RFLP analysis, 0.5 μg of DNA was amplified with unlabeled primers, and the PCR products were digested with Fnu4HI (DXYS15) or StuI (DXYS77) and were loaded onto a 3% NuSieveTM gel (FMC BioProducts) mixed with a standard agarose gel (3:1). In addition, PCR analysis was also carried out for six loci on the Y-differential region (Fig. 2).

The patient was heterozygous for DXYS85 with a paternally derived 74 bp peak and a maternally derived 78 bp peak; the ratio of area under curve between the two peaks was 1:0.98, indicating that DXYS85 was present in a single copy on both the X chromosome and the rearranged Y chromosome. Unfortunately, informative polymorphisms were not obtained for the remaining six pseudosutosomal loci. The six loci on the Y-differential region were detected in the patient.

## DISCUSSION

The rearranged Y chromosome had a partial deletion of the PAR1 and a partial duplication of Yq, and the karyotype was determined as 46,X,?i(Y)(q10).ish der(Y)(Yqter→Yp11.3::Yq11.2→Yqter)(DAZ++,DYZ3+,SRY+,SHOX–). To our knowledge, such a rearranged Y chromosome has not been reported previously. There are two possible mechanisms for the generation of the rearranged Y chromosome during paternal meiosis (Fig. 4). One possible mechanism is that crossing-over on the PAR1 between SHOX and DXYS85 (a normal event) and chromosomal breakage on Yq proximal to DAZ (an abnormal event) occurred and, subsequently, chromosomal reunion took place between the crossing-over point on the PAR1 and

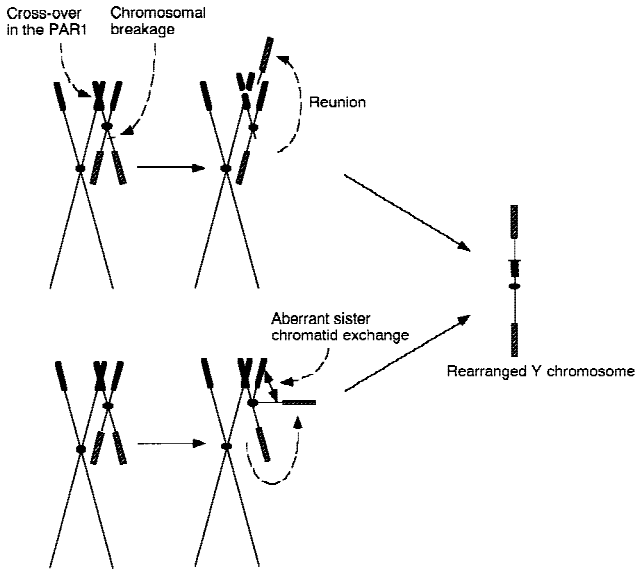


Fig. 4. A schematic representation of the generation of the rearranged Y chromosome. The thick lines indicate the short arm pseudoautosomal region (PAR1), the thin lines indicate the gender differential regions, and the striped segments indicate the Y heterochromatic region. It is inferred that the rearranged Y chromosome has been formed by chromosomal reunion between the crossing-over point on the PAR1 and the breakpoint on Yq (**upper panel**), or by aberrant sister chromatid exchange between the PAR1 and the proximal part of Yq (**lower panel**).

the breakpoint on Yq. The other possible mechanism is that aberrant sister chromatid exchange occurred between the PAR1 and the proximal part of Yq.

The structural determination provides two implications for the phenotypic effects of the rearranged Y chromosome. First, the abnormal Y chromosome lost *SHOX* but retained other genes or regions of known phenotypic effects [reviewed by Vogt et al., 1997]. This would explain why borderline short stature is the sole discernible abnormality of this boy, although his height may also be subject to the statural effect of possible duplication of the Y-specific growth gene(s) postulated on proximal Yq [Ogata et al., 1995]. Indeed, it has been suggested that *SHOX* haploinsufficiency results in borderline short stature in the absence of overt Léri-Weill dyschondrosteosis characterized by Madelung deformity and mesomelia [Kosho et al., 1999]. Furthermore, because the development of UTS skeletal abnormalties in *SHOX* haploinsufficiency seems to depend primarily on the skeletal maturing effect of gonadal estrogens [Kosho et al., 1999], this would account for the lack of Turner skeletal features in this prepubertal boy. Second, the rearranged Y chromosome lacked roughly the distal half of the PAR1. In this regard, it has been reported that synaptic formation usually begins in the telomeric region [Chandley et al., 1984], and that a 46,Y,del(X)(p22.32) male is infertile because of impaired gender-bivalent formation between the normal Y chromosome and the del(X)(p22.32) chromosome that retains pure telomere sequences but is lacking pseudoautosomal sequences [Mohandas et al., 1992]. Thus, the abnormal Y chromosome may result in spermatogenic failure due to defective X-Y pairing and recombination in the PAR1, because it is missing pure telomere sequences and roughly half of pseudoautosomal sequences. In addition, the large Yq segment translocated onto the distal Yp could also disturb the X-Y pairing and recombination.

In summary, structural analysis was carried out for a rare rearranged Y chromosome, providing indications for genotype-phenotype correlation. The present study, therefore, represents the importance of structural determination in the assessment of clinical effects of rearranged Y chromosomes.

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